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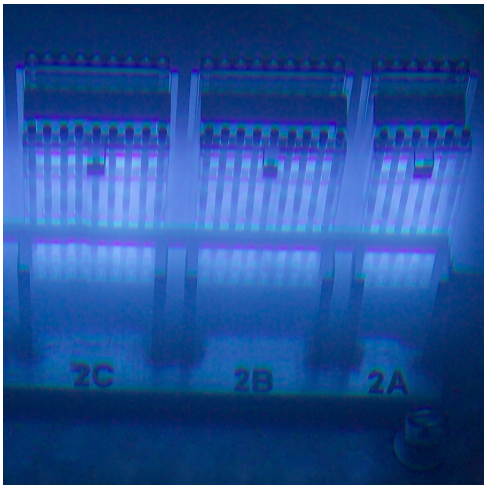
Alanine Dosimetry Accurately Determines Radiation Dose in Nonhuman Primates



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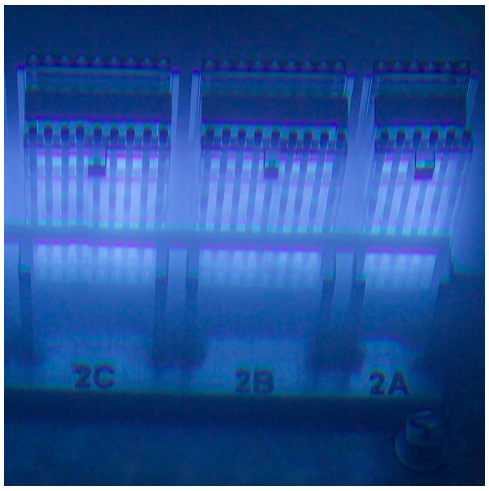

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Contents

Abstract

The unique capabilities of the Armed Forces Radiobiology Research Institute (AFRRI) cobalt-60 (^{60}Co) gamma (γ)-photon irradiator were used for bilateral whole-body irradiation of the nonhuman primate (NHP) *Macaca mulatta*. In this study, 6.5-Gy radiation was delivered at 0.4 Gy/min to 24 male NHPs 7–10 years of age weighing 7–14 kg in order to evaluate the (a) pharmacokinetics and pharmacodynamics (PK/PD) of ciprofloxacin (CIP) in an irradiated nonrodent large-animal species phylogenetically close to man, (b) utility of CIP in managing postirradiation infection related to bacterial translocation from the alimentary canal, and (c) side effects of CIP in acutely irradiated NHPs. This dose was chosen from published and unpublished studies indicating 6.5 Gy (a) approximated the $LD_{50/30}$ for NHPs and (b) resulted in severe hematopoietic system injury with increased risk for death from sepsis. For irradiation, an acrylic plastic container was constructed to comfortably restrain large NHPs that were 60–80 cm in length. Three acrylic plastic cylinders (7, 10.1, and 12.6 cm in diameter) were used in conjunction with alanine pellets to estimate the dose to the mid-abdominal target area of various sized NHPs. In addition, measurements of radiation dose to the bone marrow in each of four different-sized femurs were made by insertion of alanine film strips into the femoral cavity of bones obtained from euthanized animals. While the mid-abdominal area received 6.5-Gy radiation, doses to femoral marrow were presumptively 10% higher than to the core of NHPs, based on femoral cavity measurements. This report emphasizes and describes the accuracy and precision of alanine dosimetry, radiation field characterization and use, and the special restrainer and phantoms. In this Institutional Animal Care and Use Committee (IACUC)-approved study, all 24 NHPs survived 6.5 Gy due to (a) accurate and precise dosimetry and (b) supportive care provided to the irradiated animals.

Support for this work was provided by National Institute of Allergy and Infectious Diseases (NIAID) contract #Y1-A1-4827-01 and by AFRRI project #RAB3AG-1.

Introduction

The nonhuman primate (NHP) *Macaca mulatta* (rhesus macaque monkey) is an animal of choice for evaluating clinically beneficial or toxic agents because of its phylogenetic "closeness" to man. Indeed, in the Food and Drug Administration (FDA) promulgation of the animal efficacy rule and because of its behavioral, physiological, and hematopoietic similarities to man, it is a favored large animal species for evaluating substances such as antibiotics that may have clinical value in managing sequelae (e.g., sepsis) induced by whole-body irradiation injury.

Compared to whole-body irradiation of small animals such as mice, generally considered homogenous for purposes of radiation dose estimations, irradiation of *M. mulatta*, especially large members of the species, presents challenges in providing accurate estimates of dose deposition to target tissues (alimentary canal, e.g., gut; and hematopoietic-cell-containing bones of the body, e.g., femur). Radiation doses reported as whole-body for NHPs make unwarranted assumptions of deposition in radiosensitive targeted tissues. Factors contributing to difficulties in irradiating large animals include the (a) nonuniform distribution of radiosensitive tissues throughout the body, (b) nonuniform size of animals within treatment groups, and (c) physical location of radiosensitive proliferative tissues of the animal at the time of radiation exposure.

The purposes of this report are to describe the (a) rationale for choosing the radiation dose given to NHPs, (b) AFRRI ^{60}Co source at the time of NHP irradiation, (c) characteristics of a custom-designed acrylic plastic restraining module for irradiating large NHPs, (d) alanine dosimetry in water-containing acrylic plastic phantoms prior to irradiation of the NHPs, and (e) calibration and uncertainties of the alanine/electron paramagnetic resonance (EPR) dosimetry.

Materials and Methods

Previous studies (Broerse, *et al*. 1978; Eldred, *et al*. 1953; Eldred, *et al*. 1954; Eltringham, pers. comm.; Henschke, *et al*. 1957) reported survival responses of rhesus macaques after different radiation qualities. For example, Eldred and coworkers (Eldred, *et al*. 1953; Eldred, *et al*. 1954) used 250 kVp x-rays with a half-value layer (HVL) of 1.7 mm Cu to irradiate 37 nonhuman primate (NHP) at a rate of 13.7 R/min. The estimated $LD_{50/30}$ was 600 R. Henschke and Morton (1957) also used 250 kVp x-rays with an HVL of 1.7 mm Cu operated at 22 R/min. The $LD_{50/30}$ based on a population of 110 NHPs that included both genders was 530 R ± 21 R. The interpolated $LD_{20/30}$ and $LD_{80/30}$ were approximately 410 R and 700 R, respectively. In Eltringham's work (pers.comm.) NHPs were given 55 rad/min of ^{60}Co γ-photons. The $LD_{50/30}$, based on a population of 90 NHPs and as determined by the AFRRI statistician, was 639 rad. The calculated $LD_{20/30}$ and $LD_{80/30}$ were approximately 565 rad and 722 rad, respectively.

In the reports noted above, NHPs were not provided supportive therapy subsequent to irradiation. The $LD_{50/30}$ was 525 rad in a bone marrow transplantation study where 16 NHPs were given 300 kVp x-rays (HVL of 3 mm Cu) at 28 rad/min followed by support (Broerse, *et al*. 1978). Haigh and Paterson (1956) determined the $LD_{50/30}$ for 44 NHPs (22 of each gender) as 570 R ± 25 R using 250 kVp x-rays with an HVL of 1.6 mm Cu at 3 R/min. Dalrymple, Lindsay, and Ghidoni (1965) noted an $LD_{50/30}$ for 104 NHPs (52 of each gender) irradiated to 670 rad ± 20 rad with 2-MeV x-rays (HVL of 7.5 mm Pb) at a rate of 10.7 rad/min. The NHPs in all the above-mentioned studies were 2–4 years of age and weighed 2–4 kg.

In rhesus NHPs, 400–800 rad produces signs and symptoms of injury broadly associated with hemopoietic failure. The term "hemopoietic syndrome" defines the range of sequelae related to severe injury to bone marrow cell populations. Death from hematopoietic injury in irradiated rhesus NHPs is noted to occur between 10 and 23 days after exposure (Dalrymple, *et al*. 1965; Eldred, *et al*. 1953; Eldred, *et al*. 1954). One of the first life-threatening sequelae seen after these radiation doses and prior to death (and at *post mortem*) is infection, generally of an endogenous origin arising from injury to the large bowel and skin as well as from oral facial ulcerations. The two principal portals of bacteria resulting in sepsis in rhesus NHPs given 400–800 rad are the large bowel and oropharyngeal-facial area. Hemorrhagic lesions in the tissues mentioned also accompany infection. Some bacteria found in previous investigations in tissues and organs after irradiation included *Escherichia coli*, *Streptococcus pneumoniae*, *Pseudomonas* sp., *Proteus* sp., *Staphylococcus* sp., and *Streptococcus* sp. (Allen, *et al*. 1960; Dalrymple, *et al*. 1965; Haigh, *et al*. 1956; Schlumberger, *et al*. 1954; Wise, *et al*. 1968). Under normal conditions, these bacteria are nonpathogenic inhabitants of the alimentary canal but, in immunocompromised situations, can result in opportunistic infections.

Radiation dose selection

Taken together, these data support the use of 6.5 Gy cobalt-60 (^{60}Co) gamma (γ)-photons to induce hematopoietic injury and sepsis making that dose useful for evaluating the (1) pharmacokinetics and pharmcodynamics (PK/PD) of ciprofloxacin (CIP) in irradiated NHPs, (2) utility of CIP in managing postirradiation infection related to bacterial translocation from the alimentary canal and (3) side effects of CIP in acutely irradiated NHPs. This radiation dose approximates the $LD_{50/30}$ of the unpublished study performed by Eltringham as

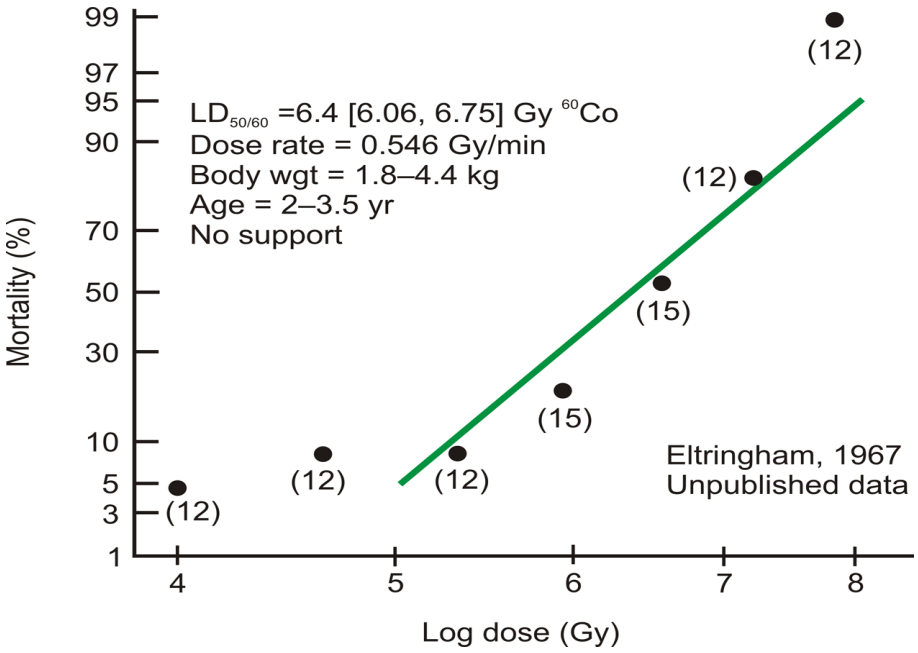

Figure 1. Mortality of ^{60}Co γ-photon irradiated male rhesus monkeys.

shown in figure 1 (Eltringham, pers. comm.) and Dalrymple *et al*. (1965).

Animal care

Male *M. mulatta* weighing 7–14 kg and 7–10 years of age were obtained from the AFRRI and Walter Reed Army Institute of Research (WRAIR) issue pools. The NHPs had been part of approved dengue and yellow fever immunization protocols 2–4 years prior to irradiation. The NHPs were housed singly in sanitized stainless steel cages in holding rooms provided with 10 changes/hr of 100% fresh air, conditioned to 22ºC and a relative humidity of 50% ± 20%. They were maintained on a 0600 light–1800 dark full-spectrum light cycle. Commercial primate chow supplemented with fruit, vegetables, and autoclaved water *ad libitum* was provided. Prior to irradiation, the NHPs were anesthetized with 10 mg/kg of ketamine (Ketaset®, Fort Dodge Laboratories, Fort Dodge, IA) given intramuscularly, placed in the restraint device described below, and allowed to regain consciousness.

All procedures involving the NHPs were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) and received second-tier animal care and use review and approval. All NHPs were monitored telemetrically 24 hr/day 7 days/week and visually at intervals of 4–6 hr. This research was conducted according to principles outlined in the *Guide for the Care and Use of Laboratory Animals* of the Institute of Laboratory Animal Resources, National Research Council.

Subsequent to acclimation in the veterinary facility, the NHPs were surgically implanted with telemetric devices for continuous monitoring of body temperature, electrocardiogram (ECG), and respiratory function. The telemetry data are the subject of a future report. Physical activity monitoring of the NHPs was performed by Doppler microwave sensors placed above experimental cages. A minimum of one month after ensuring healing of

the telemetric implant site, 15 mg/kg and 30 mg/kg CIP were provided orally to each of two groups of eight nonirradiated NHPs twice daily (0600 and 1800) for three consecutive days for the purposes of PK/PD determinations. Phlebotomies for these determinations were performed at 15 min, 30 min, 1 hr, 2 hr, 4 hr, 6 hr, 8 hr, and 12 hr after the last CIP dosing. At a minimum of 30 days after these procedures, the NHPs were given 6.5 Gy at 0.4 Gy/min. Four days after irradiation, the same CIP doses were given orally to the same animals and phlebotomies performed as noted.

The data from these PK/PD studies will be presented in a future report. In addition, irradiated animals continued to receive twice daily doses of CIP for 21 consecutive days after irradiation. During this period, on days 7, 10, 14, 17, 21, and 25 after irradiation, oral and rectal cultures were taken for microbiologic identification and susceptibility studies; bone marrow cells were harvested for clonogenic determinations; and blood was drawn for hematology and clinical chemistries. Data from these studies will also be presented in a future report. Support for irradiated NHPs was given as needed and included fluids given intravenously, soft palatable food, Tylenol™ given orally for temperatures greater than 103°F, and antibiotics for bacteria resistant to CIP following T > 103°F.

AFRRI ^{60}Co γ-photon radiation source

AFRRI offers unique radiation sources and source configurations for irradiating animals. Of these sources, the ^{60}Co high-dose rate irradiator provides γ-photon irradiation from cassettes contained within two sets of moveable carriages, each containing up to 16 radioactive source rods permitting unilateral or bilateral irradiation. Dose rates can be varied by altering the number of source rods or the source-to-target distance (Carter and Verrelli 1973). Doses of γ-radiation for dosimetry and irradiation of NHPs reported here were obtained from ^{60}Co rods #1–7, 9–24, and 26–32 (old rods and cassettes circa 1989). The activity of the AFRRI ^{60}Co facility during the NHP irradiations was 33,000 curies (^{60}Co source rods). NHPs were given 6.5 Gy bilaterally at a rate of 0.4 Gy/min to the abdominal core to enhance bacterial translocation from the gut as is described below. NHPs in this study were irradiated in a six-month period from May to November 2005.

Physical characteristics of the acrylic plastic radiation restrainer

Figure 2 shows the acrylic plastic (Plexiglas™) radiation restrainer facilitating irradiation of NHPs. The side walls, top, and bottom of the restrainer were constructed of 0.5-cm acrylic plastic stock material. The height of the restrainer was 89 cm, established by the length of the tallest NHP in the experimental protocol. The width was 49 cm, and depth, front to back, was 69 cm. A girth hole in the shelf, which could be used as a seat, was set at a diameter of 13 cm; a neck slot was set at a diameter of 10.5 cm.

Dosimetry

Dosimetry was performed using the alanine/electron paramagnetic resonance (EPR) system (Bruker GmbH, Germany; ASTM 1994). The calibration of the system was directly traceable to the National Institute of Standards and Technology (NIST), and its accuracy was additionally verified by intercomparisons with the National Physics Laboratory (NPL) in the United Kingdom. To determine dose rates in the abdominal cores of different sizes of NHPs, three cylindrical phantoms made of polymethyl methacrylate (PMMA) and filled with water to simulate tissue equivalency were used (figure 3). The phantoms measured 35 cm high, with outer

Figure 2. The acrylic plastic restrainer box used for radiation field mapping and irradiation of NHPs (May to November 2005). Slotted sides for adjusting the "seat" accommodated the animal's height. Four knob-type plastic screws adjust and tighten the "seat" from front to back. Six black nylon t-bar cleats were used to secure animals with 3/8" nylon rope. Fabric strips were used to prevent chafing on arms and legs. White cords on each side aided in lifting the box. A grooved neck plate at the top of the restrainer was adjustable to fit different sizes of collars. Animals were fitted and acclimated to collars prior to placement in the restrainer. A vertical sliding door (not shown) was slid into place (a) for dosimetric determinations, (b) to secure animals within the box during irradiation, and (c) to provide stability of the box. The first groove was placed at a height of 53.1 cm while the second through the sixth grooves were placed at 4-cm intervals to adjust for differences in animal height. Adjustments of support and neck grooves allowed animals to fit in the restrainer box.

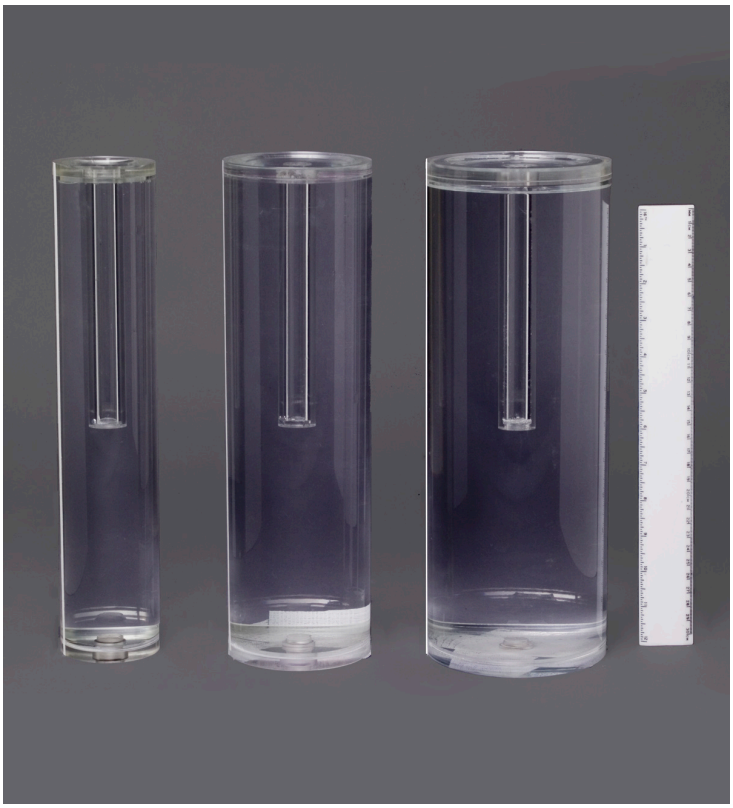

Figure 3. Water-filled PMMA phantoms used to determine dose rates for NHPs of different diameters. The three phantoms, whose diameters of 7–12.6 cm spanned the diameters (ischial-ischial distance) of all NHPs, were used to construct a dose-rate calibration curve. The curve was used to establish the dose for each NHP.

diameters of 7.0, 10.1, and 12.6 cm, respectively. The phantoms had sleeves from their tops to their cores, whose inner diameters matched precisely the outer diameter of vials containing alanine pellets.

Alanine pellets (Gamma Service, Dresden, Germany; Lot T020604) with mass of 65.5 ± 0.5 mg were used for dose rate measurements. Prior to use, the pellets were stored in a desiccator over phosphorus pentoxide, which provided 0% relative humidity (Sleptchonok, *et al*. 2000). The pellets were conditioned to the humidity of the ambient environment before use (Nagy, Puhl, *et al*. 2000) and were irradiated in the phantoms in plastic vials purchased from NIST (figure 4). The vial height was approximately 30 mm; the outer diameter was 12 mm, while the inner diameter allowed 0.2 mm of space for the 4.9 mm diameter of the pellets (Nagy and Desrosiers, 1996). Walls of the vials were thick enough to provide electron equilibrium. Each vial contained four alanine pellets. The phantoms were irradiated by placing them inside the enclosed restrainer box used for NHP irradiation (figures 5 and 6).

During irradiation, the NHPs were positioned in such a way that most of the animal's body was irradiated through the thinner upper parts of the side walls of the chamber. That eliminated errors from the irregular attenuation of γ-photons by the grooves (used for shelf adjustments) in the middle part of the restrainer's walls. In dosimetric irradiations, the phantoms were positioned accordingly.

The degree of scattering by this container was small at the energy of the γ-quanta produced by ^{60}Co (1.25 MeV). In the horizontal plane, the phantoms were centered in the box that, in turn, was centered on the exposure table. The accuracy of the phantom positioning in the box was deliberately chosen as ± 1 cm from the center to account for inaccuracy of the positions and movements of the NHPs. Results of previous field mappings for irradiation of mice with similar positions of the ^{60}Co source rods suggest that the variations of the radiation field intensity over the areas occupied by the NHPs did not exceed ± 1%, although no special mapping was made for the field in this restrainer box.

To obtain precise dose rate values, alanine pellets were irradiated inside the phantoms in the restrainer box for exactly measured periods of time in the range of 6–8 hr. As the dose rates were approximately 0.4 Gy/min, the total doses to the pellets were in the range of 150–200 Gy. The rod-containing elevators were set at 340 cm from the center of the table, in line with the expected position of the animal's body. This geometry provided the exposure rate in the reference point of the facility of 48.1 ± 0.2 R/min over the period of mapping. There were two replicate runs for each phantom size. All the irradiations for dosimetry were performed during a two-week period (May 7–17, 2005) prior to irradiation of the NHPs.

EPR measurements of signals from irradiated alanine pellets were performed at least one day after the end of the irradiation to eliminate a small (<1%) but measurable decrease in signal intensity during the first hour after exposure (Nagy, *et al*. 1996). Measurements were performed on a Bruker e-Scan EPR spectrometer specially designed for highly accurate intensity measurements necessary in alanine dosimetry.

A critically important component of this instrument is an adjacent reference sample (Nagy, Sleptchonok, *et al*. 2000). This immobile sample rests in a remote part of the instrument's sensitive area during the whole measurement session, and its signal (insignificantly overlapping the alanine signal) is recorded automatically each time the alanine

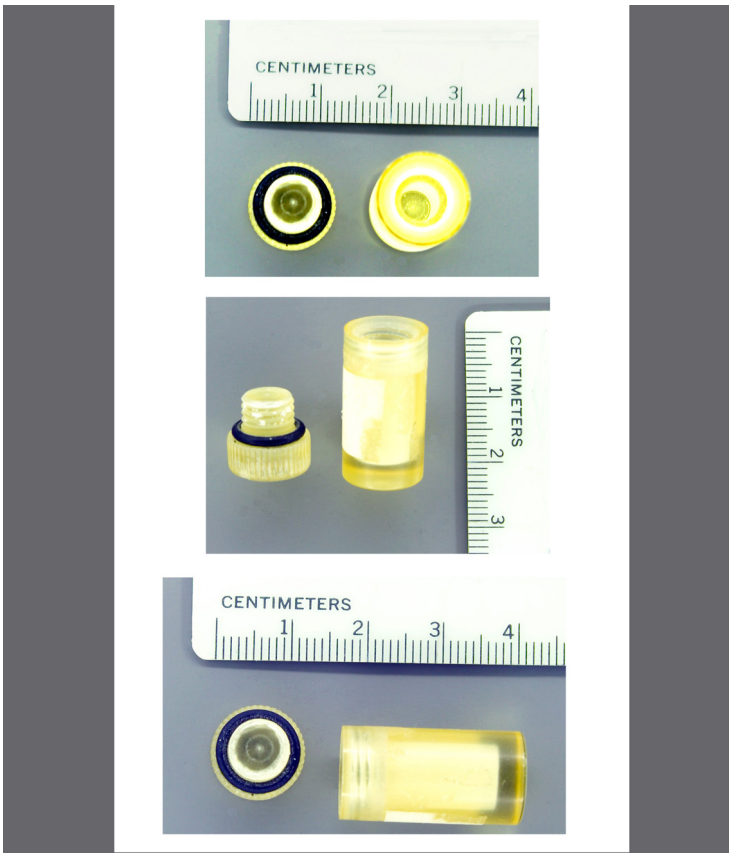

Figure 4. Vials for alanine pellets used at NIST and AFRRI for irradiations under electron equilibrium conditions. (Photos provided by T.B. Elliott, PhD, and M.O. Shoemaker, PhD.)

Figure 5. The restrainer used in dosimetric measurements and for irradiation of NHPs in the AFRRI ^{60}Co whole-body irradiator. The restrainer's larger sides, identified by white ropes, face a north-south orientation. A PMMA cylinder used to simulate the body of an NHP and to hold the alanine dosimeter package is placed on the first shelf within the restrainer. The restrainer is centered on the table surface. The restrainer is located on a table fitted over a pool of water midway between two carriages containing the ^{60}Co source rods. The ^{60}Co rods are stored in cassettes at the bottom of the 15-foot-deep pool when not used.

signal is recorded. As small changes in the instrument sensitivity due to temperature or power instability are likely to affect both signals to the same degree, the ratio of the intensities, used as the measure of the alanine signal, is almost insensitive to these interferences. This makes it possible to achieve much higher accuracy for dose determinations than with traditional EPR spectrometers.

Software built into the spectrometer was used to automate the measuring process and to determine radiation doses. An alanine response temperature coefficient of 0.14% per degrees Celsius (Nagy, Puhl, and Desrosiers 2000), provided by the software by default, was used to correct for small differences in the temperature of irradiation at AFRRI and NIST.

Doses to the alanine pellets irradiated in the phantoms were determined using a calibration curve constructed with a standard alanine calibration set (PV040220A) purchased from NIST. The declared accuracy of the doses given to the dosimeters was 1.7% at 2σ (95%). These doses to the pellets were provided by the U.S. primary national standard ^{60}Co source located at NIST. Thus, the AFRRI EPR spectrometer was calibrated directly against the national standard source.

Accuracy of this calibration curve was confirmed by a subsequent blind intercomparison between the two laboratories. In that intercomparison, unknown radiation doses, to the same set of irradiated pellets, were measured in parallel at AFRRI using the calibration curve and at NIST. Differences between the AFRRI and NIST results ranged for different pellets from +0.15% to –1.56%.

Accuracy of the calibration curve was also confirmed by an additional check against the national standard ^{60}Co source of the NPL, currently regarded as the most reputable alanine dosimetry service in the world.

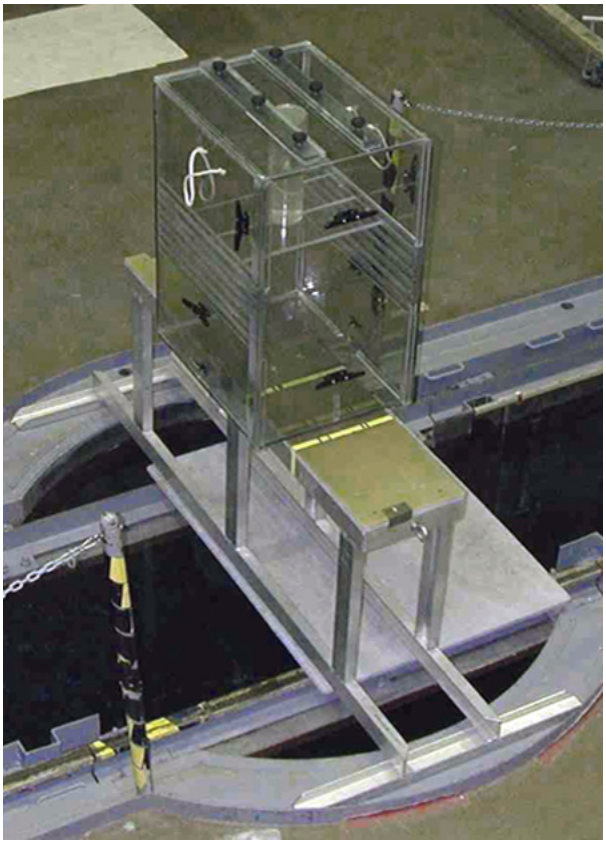

Figure 6. A high-angle view of the acrylic plastic NHP-restraint box centered on the table in the AFRRI ^{60}Co whole-body irradiator. The restrainer is secured to the table during animal irradiation with elastic straps. The table is immobolized by aluminum beams, secured to the bottom of the table, that contact the arcs located on the top of the pool. A water-filled PMMA cylinder used for dosimetry is located between the white ropes used for carrying the restraint box.

Table 1. Doses and dose rates to water in phantom cores at the time of irradiation.

Small phantom (diameter: 7.0 cm)						
	In-vial position	**Dosimeter number**	**Dose (kGy)**	**Dose rate (Gy/min)**	**Mean dose rate (Gy/min)**	**Relative standard deviation**
Replicate #1, irradiation time: 8:01:04 (481.1 min)						
	top	4	0.215	0.4464	0.4483	0.30%
		3	0.216	0.4494		
		2	0.216	0.4491		
	bottom	1	0.216	0.4484		
Replicate #2, irradiation time: 8:00 (480.0 min)						
	top	4	0.216	0.4499	0.4511	0.30%
		3	0.216	0.4502		
		2	0.217	0.4515		
	bottom	1	0.217	0.4528		
Interrun mean					0.4497 kGy	
Interrun difference					0.61%	
Medium phantom (diameter: 10.1 cm)						
	In-vial position	**Dosimeter number**	**Dose (kGy)**	**Dose rate (Gy/min)**	**Mean dose rate (Gy/min)**	**Relative standard deviation**
Replicate #1, irradiation time: 8:00 (480.0 min)						
	top	4	0.207	0.4306	0.4341	0.56%
		3	0.209	0.4353		
		2	0.209	0.4344		
	bottom	1	0.209	0.4362		
Replicate #2, irradiation time: 8:00 (480.0 min)						
	top	4	0.208	0.4335	0.4334	0.42%
		3	0.207	0.4309		
		2	0.209	0.4352		
	bottom	1	0.208	0.4341		
Interrun mean					0.4338 kGy	
Interrun difference					0.16%	
Large phantom (diameter: 12.6 cm)						
	In-vial position	**Dosimeter number**	**Dose (kGy)**	**Dose rate (Gy/min)**	**Mean dose rate (Gy/min)**	**Relative standard deviation**
Replicate #1, irradiation time: 8:00 (480.0 min)						
	top	4	0.204	0.4243	0.4260	0.44%
		3	0.205	0.4277		
		2	0.204	0.4245		
	bottom	1	0.205	0.4276		
Replicate #2, irradiation time: 6:00 (360.0 min)						
	top	4	0.148	0.4121	0.4141	0.52%
		3	0.148	0.4124		
		2	0.150	0.4159		
	bottom	1	0.150	0.4161		
Interrun mean					0.4201 kGy	
Interrun difference					2.82%	

Results

Table 1 shows results of dose rate measurements.

Mean dose rates for two replicate experiments with the small and medium phantoms differed by 0.61% and 0.16%, respectively. The discrepancy for the large phantom was 2.82%. No additional experiments to reconcile differences for the large phantom were performed, and the results of the two replicates were averaged. The largest error possibly introduced by averaging the data is likely less than 1.4%.

Dose rates

The dose rates within the three different-sized phantoms were approximated by an empirical function. Figure 7 shows fits to the average dose rates for each phantom.

The fit can be used to determine dose rates to a phantom of any intermediate size, which, in turn, can be used as an estimate of the dose rate in the abdominal core of an NHP of the corresponding size.

Thus, calculation of the exposure time for each NHP includes a measurement of the animal's dimension (ischial to ischial distance) as oriented to the radiation beam (the NHPs were oriented with their sides to the ^{60}Co rod sources). Exposure times for the NHPs varied from 14.6–16.28 min. Other operations involved in calculations of the exposure time are (a) determination of the dose rate to water in the phantom of the corresponding diameter using the fit, (b) application of the correction for ^{60}Co decay between the field mapping and actual animal irradiation, (c) application of the correction for the small difference in the γ-photon absorption between water and soft tissue, and (d) application of a correction for untimed irradiation during the rise and fall of the ^{60}Co rod sources (approximately 1%).

Dose uncertainties

Table 2 lists the main contributions to the uncertainty in doses given to NHPs. The values in the right column represent single standard deviations divided by the corresponding values (i.e., relative standard deviations).

As these sources of uncertainties are independent, the combined uncertainty is obtained by summing the corresponding relative standard deviations in quadratics. Thus, the overall uncertainty of 2.1% for 1σ or 4.2% for 2σ (the latter corresponds approximately to the confidence interval at 95%) can be determined.

It should be noted, however, that the calculated exposure times and resulting reported doses correspond to the abdominal cores of the NHPs. These doses are lower than those in areas located closer to skin because of the natural attenuation of the γ-photons. Furthermore, it is assumed that the "core point"

Table 2. Components of uncertainties of the dose given to the abdominal cores of NHPs.

Uncertainty component	Relative standard deviation
Uncertainty of EPR measurements of doses to alanine	0.015 (1.5%)
Uncertainty of fit relating phantom size with dose rate	0.01 (1%)
Uncertainty of selected dose rate due to uncertainty in the monkey size measurement	0.01 (1%)

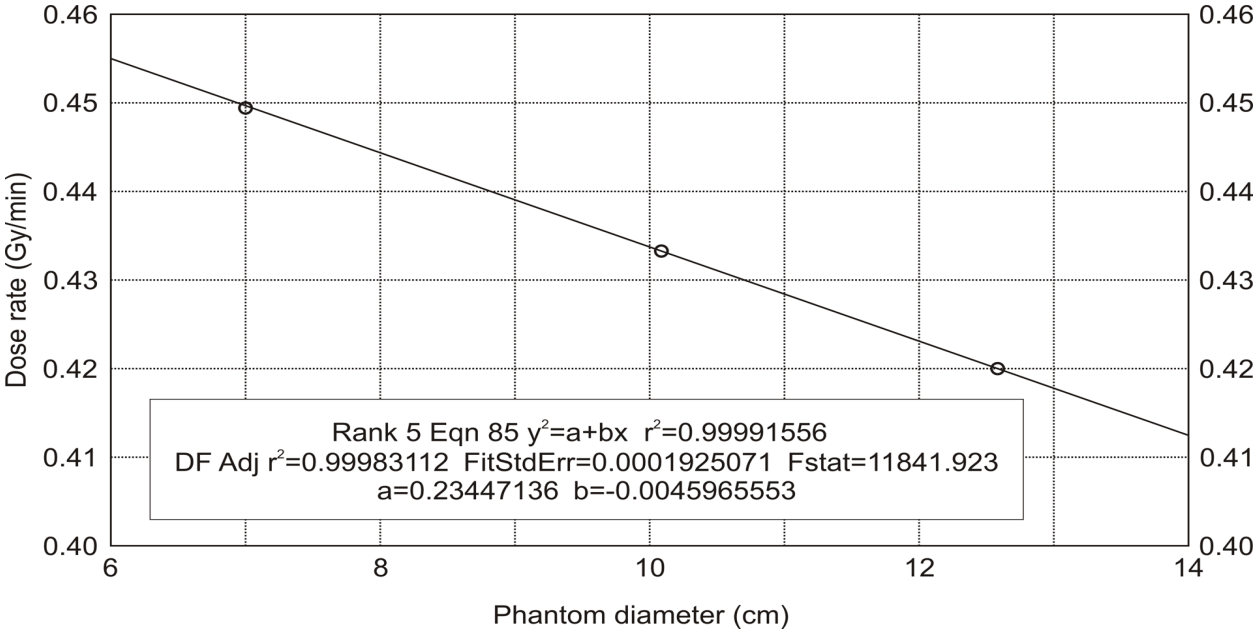

Figure 7. Dependence of radiation dose rate to water relative to the diameter of the phantom.

of interest is within the abdomen and that the animals are oriented with their sides to the sources. As such, bones would not interfere with the beam path, passing entirely through soft tissue. Parenthetically, it would be far more complicated to precisely calculate the dose to a specific region of a body if irregular bones are in the exposure field.

Dose delivered to femurs

In other work, we estimated the radiation dose to the marrow of femurs obtained from NHP cadavers. In this effort, four femurs were irradiated adjacent to the small, 7-cm diameter phantom. The phantom and the femurs contained alanine films (Kodak Biomax). The width of the films was 4 mm, the length of the alanine-containing portion 47 mm. Figure 8 shows the setup.

After irradiating the bones to 1 kGy (a dose necessary because of the lower sensitivity of alanine films as compared with that of alanine pellets), relative doses to the films were measured using the Bruker e-Scan spectrometer (as with the pellets), but fitted with a special film holder. At 1 kGy, alanine response to the dose is still linear. Therefore, the ratio of the alanine signal in the core of the phantom to the alanine signal in the bones provides the ratio of the corresponding dose rates. Table 3 lists the results normalized, for convenience, to the dose rate in the core of the mid-sized (10.1 cm), rather than the small, phantom using the fit. The femurs identified in table 3

Table 3. Relative dose rate in femurs obtained from NHP cadavers.

NHP bone origin	Dose rate in the bone cavity relative to the dose rate in the core of the mid-sized phantom (10.1 cm in diameter)
A (female)	108%
B (female)	113%
C (male)	110%
D (male)	108%

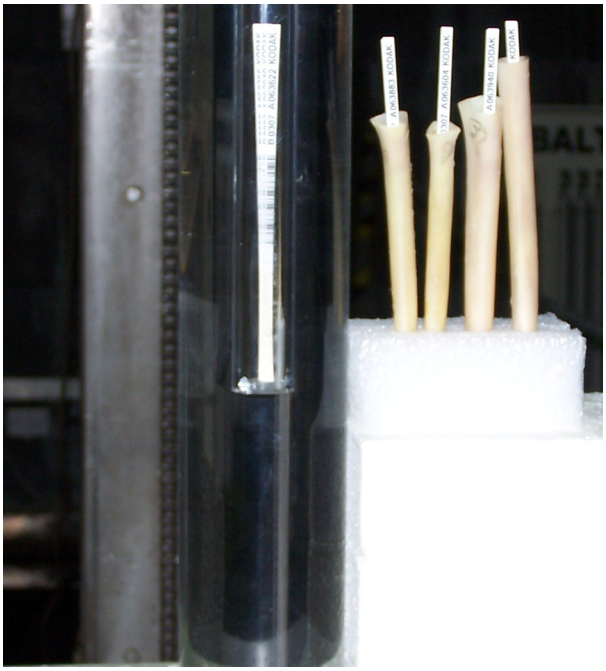

Figure 8. A view of the system employed to estimate radiation dose inside the marrow cavity of four NHP femurs. Four dry femurs of different diameters, devoid of any tissue but containing alanine strips, were placed in line with the small-sized phantom (7 cm in diameter) containing similar alanine strips. The plane in which all the alanine films were located was perpendicular to the direction of the radiation beam. Foam blocks were used as a support.

are from four separate NHPs.

Thus, doses to femoral bone marrow were, on average, 10% higher than those to the core of the mid-sized phantom. There is no clear correlation between the dose rates and the apparent dimensions of the bones. This is not surprising because the femoral bone thickness is very irregular and the alanine-containing zone of the film is long. Moreover, even if such a correlation was observed, it would be of limited usefulness because the wall thickness of the bone is different in different parts of the femur and, accordingly, different portions of the bone marrow receive different radiation doses. It can be deduced from this experiment, with a reasonable degree of reliability, that doses to femoral marrow are roughly 10% higher than doses to the abdominal core of a mid-sized monkey.

Discussion

Survival or mortality subsequent to ionizing radiation depends upon a number of physical factors. Principle physical components of radiation exposure impacting biologic responses include the inherent nature (quality) of the radiation and total dose and dose rate. In our work we used 1.25-MeV photons from a ^{60}Co γ-photon source and delivered 6.5 Gy at 0.4 Gy/min to the mid-abdominal area. These attributes were selected based upon the published efforts of Dalrymple et al. (1965) and the unpublished work of Eltringham (pers. comm.). They employed radiation sources resulting in comparable $LD_{50/30}$ rad-doses (670 vs. 639) even though the rad-dose rates were dissimilar (10.7 vs. 55 Gy/min). The higher $LD_{50/30}$ rad-dose was achieved with 2-MeV x-ray photons (Dalrymple, *et al*. 1965) while the lower value was obtained with 1.25-MeV photons (Eltringham, pers. comm.).

It is noteworthy that the National Institute of Standards and Technology (NIST) and the National Physics Laboratory (NPL) provide radiation source calibrations with alanine directly in terms of absorbed dose to water, not in terms of exposure as do most other calibration institutions. Conversion of exposure (which is essentially a charge produced in air by radiation) into absorbed dose (energy absorbed inside a specific absorbing object) involves a number of experimentally measured parameters and difficult-to-verify assumptions, which makes it prone to inaccuracies.

By contrast, calibration with alanine against the national standard sources requires only two corrections: one for ^{60}Co decay with a precisely known half-life of 1925.23 days (Nagy, Puhl, *et al*. 2000) and another for differences between the mass energy absorption coefficients for water and soft tissue, which is small (<1%) and reasonably well-known (Woods, *et al*. 2004).

In our work, dosimetry was accomplished using appropriate-sized acrylic-plastic phantoms and alanine pellets and film-strips exposed in the radiation field and read in a Bruker e-Scan spectrometer. The work reported earlier (Allen, *et al*. 1960; Broerse, *et al*. 1978; Dalrymple, *et al*. 1965; Eldred, *et al*. 1953; Eldred, *et al*. 1954; Eltringham, pers. comm.; Haigh, *et al*. 1956; Henschke, *et al*. 1957; Schlumberger, *et al*. 1954) employed Victoreen R-meters, Siemens Universal thimble chambers (Henschke, *et al*. 1957), and EPR with alanine (Dalrymple, *et al*. 1965). The latter was one of the very first uses of alanine as a dosimetric material at the time when the method was still in its infancy. Many presently used techniques for enhancing the accuracy of the method were not known at that time. In our effort, stringent measures were taken to ensure that alanine dosimetry was used with all the available knowledge about this method properly taken into account. In addition, alanine dosimeters used at AFRRI to construct calibration curves were traceable to national standard sources at NIST and the NPL. Quality traces were not indicated by the earlier work (Allen, *et al*. 1960; Broerse, *et al*. 1978; Dalrymple, *et al*. 1965; Eldred, *et al*. 1953; Eldred, *et al*. 1954; Eltringham, pers. comm.; Haigh, *et al*. 1956; Henschke, *et al*. 1957; Schlumberger, *et al*. 1954).

The focus in the early work (Allen, *et al*. 1960; Broerse, *et al*. 1978; Dalrymple, *et al*. 1965; Eldred, *et al*. 1953; Eldred, *et al*. 1954; Eltringham, pers. comm.; Haigh, *et al*. 1956; Henschke, *et al*. 1957; Schlumberger, *et al*. 1954) was animal mortality and hematopoiesis as determined by constructing dose-response curves from whole-body irradiation. In summary, the delivered dose was accurately determined by alanine dosimetry with the mid-abdominal area the target site for en-

suring radiation-induced damage to the gut epithelial layer allowing for the translocation of bacteria. Further, we determined that the radiation dose to femurs was approximately 10% higher than that to the gut. This is important as depletion of hematopoietic cells in the marrow of all major long bones such as the femur would aid in bacterial translocation and sepsis, a principal aim of this work. The delivered radiation dose successfully resulted in profound neutropenia and thrombocytopenia. Following irradiation and CIP dosing, qualitative changes in the bacterial flora of the alimentary canal followed by increased resistance to management with CIP were observed.

A major component of this research was to isolate and identify major groups of bacteria from the alimentary canal. In nonirradiated NHPs, principal oral and rectal gram-negative isolates included *Escherichia coli* while gram-positive oral and rectal isolates included members of the streptococci, staphylococci, and cornybacteria genera. When the NHPs were given 6.5 Gy in the study, the incidence of gram-positive organisms increased as did the resistance of these bacteria to CIP. We did not find other gram-negative opportunistic pathogens (*Proteus* sp., *Pseudomonas* sp., and *Strepococcus pneumoniae*) in the alimentary canal as was observed by others (Allen, *et al*. 1960; Dalrymple, *et al*. 1965; Haigh, *et al*. 1956; Schlumberger, *et al*. 1954; Wise, *et al*. 1968). The specific changes in alimentary canal bacteria and their susceptibility to a panel of antimicrobials will also be the subject of a future report.

Lastly, all 24 NHPs given 6.5 Gy in this study survived the 30-day observation period. Indeed, survival for these NHPs currently ranges from 1.5–2 years postirradiation. No remarkable physiologic or pathologic findings were recorded during this extended time period even though significant blood chemistry, peripheral blood, bone marrow, and alimentary canal bacteria changes were noted during the 30-day postirradiation period. The survival recorded is ascribed in large measure to (a) the accurate dosimetric targeting and delivery of radiation and (b) clinical support abetted by continuous telemetric monitoring. These two factors permitted the evaluation of CIP for therapy of high-dose radiation-induced immunosuppression in a large animal species phylogenetically close to man.

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Acknowledgments

Support for this work was provided by NIAID contract #Y1-A1-4827-01 and by AFRRI project #RAB3AG-1. Expert assistance was provided as follows: Dr. A. Nurtaeva for radiation dosimetry; HM3 K.T. Allen and HM1 T.L. Hemphill for radiation source operation; HM2 M.A. Foriska, LT A. Germana, MAJ B. Gnade, D.L. Bolduc, Dr. J. Thakar, and V. Villa for animal handling and microbiological analysis; LTC A.K. Hildebrand for animal welfare advice; LTC J.G. Harre, MAJ C.A. Ege, and Dr. J.J. Beren for surgical procedures and veterinary care and welfare; D.E. Giraldo, Shelly Callahan, and H.D. Clinton for tissue sample analyses; TSgt S.E. Beach, SGT D.M. Lyon, CPL R. Walker, SPC N. Meadows, and SPC J.L. Kelly for animal handling and care; David Morse for photographs, Mark Behme and Patrice Bolté for graphic design, and Erica Pirrung and Frank Duffy for editorial support.

REPORT DOCUMENTATION PAGE		Form Approved OMB No.	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.			
1. Agency use only	2. Report date October 2007	3. Report type and dates covered Special Publication	
4. Title and subtitle Alanine dosimetry accurately determines radiation dose to nonhuman primates			5. Funding numbers
6. Authors V Nagy, PhD; NC Parra, CPT, DVM, USA; MO Shoemaker, PhD; TB Elliott, PhD; GD Ledney, PhD			
7. Performing organization name(s) and address(es) Armed Forces Radiobiology Research Insitute 8901 Wisconsin Avenue Bethesda, MD 20889-5603			8. Performing organization report number SP07-1
9. Sponsoring/monitoring agency name(s) and address(es)			10. Sponsoring/monitoring agency report number
11. Supplementary notes			
12a. Distribution/availability statement Cleared for public release; distribution unlimited			

13. Abstract (maximum 200 words)

The unique capabilities of the Armed Forces Radiobiology Research Institute's (AFRRI) cobalt-60 (^{60}Co) gamma (γ)-photon irradiator were used for bilateral whole-body irradiation of the nonhuman primate (NHP) *Macaca mulatta*. In this study, 6.5 Gy radiation was delivered at 0.4 Gy/min to 24 male NHPs 7–10 years of age weighing 7–14 kg in order to evaluate the (a) pharmacokinetics and pharmacodynamics (PK/PD) of ciprofloxacin (CIP) in an irradiated non-rodent large-animal species phylogenetically close to man, (b) utility of CIP in managing postirradiation infection related to bacterial translocation from the alimentary canal, and (c) the side effects of CIP in acutely irradiated NHPs. This dose was chosen from published and unpublished studies indicating 6.5 Gy (a) approximated the $LD_{50/30}$ for NHPs and (b) resulted in severe hematopoietic system injury with increased risk for death from sepsis. For irradiation, an acrylic plastic container was constructed to comfortably restrain large NHPs that were 60–80 cm in length. Three acrylic plastic cylinders (7, 10.1, and 12.6 cm in diameter) were used in conjunction with alanine pellets to estimate the dose to the mid-abdominal target area of various sized NHPs.

14. Subject terms Ionizing radiation, cobalt-60, dosimetry, radiation, NHP, ciprofloxacin, alanine pellets, whole-body irradiation			15. Number of pages 32
			16. Price code
17. Security classification of report UNCLASSIFIED	18. Security classification of this page UNCLASSIFIED	19. Security classification of abstract UNCLASSIFIED	20. Limitation of abstract

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